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EPIZOOTIOLOGIC STUDIES OF ANAPLASMOSIS IN OREGON MULE DEER[1]

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Abstract: Blood and ticks were collected from 31 mule deer *(Odocoileus hemionus hemionus)* in three bovine anaplasmosis enzootic areas of Oregon. Blood from each deer was inoculated into nonsplenectomized anaplasmosis-free calves to determine whether latent anaplasmosis was present. Each of five calves received blood from five and one received blood from six deer. Anaplasmosis did not occur in any of the calves during a 106 to 111 day post-inoculation observation period. The susceptibility of the calves was subsequently challenged with blood from an infected *(Anaplasma marginale)* carrier. All proved susceptible based on blood parasitemia, reduced packed cell volume, and reduced hemoglobin values. These observations were subsequently confirmed when the calves became seropositive to the card and complement-fixation tests.

Ticks, most of which were *Dermacentor albipictus* were collected from the deer and fed on anaplasmosis-free splenectomized calves. None of the calves became infected.

Both the card and complement fixation tests proved unreliable when conducted on mule deer serum.

INTRODUCTION

A recent survey of bovine diseases conducted in the United States by the American Cattlemen's Association placed anaplasmosis fourth among the top ten most important disease entities. This widespread disease caused by *Anaplasma marginale*, a blood parasite, is of considerable importance in Oregon and in many western, southwestern, southern and southeastern states. It is not restricted to the United States, but is also found in many foreign countries.

As a prerequisite to development of sound, economically feasible control programs and perhaps area eradication programs, greater knowledge regarding the anaplasmosis reservoir status of wildlife, especially wild ruminants, must be gained. This information is also important to elucidate the epizootiology of anaplasmosis and thereby preclude circumstantial incrimination of wildlife species which do not serve as reservoirs of infection.

Numerous species of wild ruminants have been experimentally infected with *A. marginale*. Some, such as the Columbia black-tailed deer *(O. h. columbianus)* and its crosses with mule deer *(O. h. hemionus)*, have been shown in California to be naturally infected and latent carriers.[4,5,6,7,11,12] Disease transmission between these deer and cattle commonly occurs.[5,7] Research on native white-tailed deer *(O. virginianus)* conducted in nine southeastern states demonstrated no natural infection or latent carriers in 262

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deer examined.[3] Similarly, of 49 white-tailed deer sampled in Wyoming, none were infected.[9]

The status of mule deer regarding their susceptibility to natural infection and role as latent carriers has not been adequately investigated. A trial in Wyoming conducted on 180 mule deer, blood samples collected by hunters in five areas of that state indicated infection in only one area. The authors concluded that because the incubation period in the inoculated calf was long, the infectivity of the inoculum must have been quite low and probably only one or two deer of those sampled in this area were infected.[9]

Vector transmission of anaplasmosis in the western range area of the United States also needs further study. There is little information concerning the importance of *D. albipictus* (Packard) in natural transmission of *A. marginale*. It has been reported that this tick was capable of mechanical transmission when nymphs that had fed on an infected calf were later allowed to feed on a susceptible calf.[1] The likelihood of this occurring in nature, however, would not seem to be great. Attempted hereditary transmission of *A. marginale* with *D. albipictus* was unsuccessful.[8]

The purpose of this investigation was to: (1) determine whether natural infection and latent carriers occurred among mule deer ranging in anaplasmosis enzootic areas of Oregon; (2) determine the accuracy of card (CT) and complement fixation (CF) tests conducted on mule deer serum; and (3) determine if specimens of *D. albipictus* collected from mule deer could transmit *A. marginale* to susceptible calves.

MATERIALS AND METHODS

With the assistance of the Oregon Game Commission and the Department of Fisheries and Wildlife, Oregon State University, 31 mule deer in eastern and central Oregon were shot and sampled. Eleven were collected on March 19 and 20 in the Steens Mountain area, ten on March 29 and 30 in the Ironside area, and ten on April 4 and 5 in the Silver Lake area. Ages varied from 9 months (4 deer) to 2 to 10 years (27 deer). Three were males and 28 females. All collection sites were in anaplasmosis enzootic areas. Collection was done early in the spring while deer remained in herds on winter range or were just beginning the migration to summer range. Since conditions of collecting precluded neck shooting (up to 400 m), it was not possible to collect identical amounts of blood from each deer. Adequate amounts nevertheless were collected; the least amount collected from any one deer and inoculated into a calf was 30 ml, the largest 215 ml with a mean of 92 ml. Two 250 ml vacuum bottles, one containing 1% heparin and one with no anticoagulant were used for collecting blood from each deer. All blood was injected into calves within 36 hours of collection and most samples were injected within 24 hours. Number of deer samples injected into each calf, blood volume injected, inoculation route, calf's age and collection area are shown in Table 1. Blood films prepared from deer blood were stained by the Wright's and Giesma's methods and examined by light microscopy for anaplasma bodies.

Blood clots were removed from unheparinized blood samples, and following centrifugation, serum was pipetted into glass vials after which each vial was fire sealed, identified and refrigerated at —70 C. Samples were later airmailed in insulated containers with dry ice to the Animal Parasitology Institute, Beltsville, Maryland, for CT and CF testing.

Ticks collected from each deer were placed in identified containers and also airmailed to the Animal Parasitology Institute for identification and disease transmission studies. Upon arrival, all dead specimens were discarded. Dr. R. K. Strickland, Animal Health Division, USDA-ARS, identified the species of living ticks. They were then placed on

[3] Beddell, R. M., and Miller, J. F.: A Report of the Examination of 270 White-tailed Deer, *Odocoileus virginianus*, from Anaplasmosis Enzootic Areas of Southeastern United States for Evidence of Anaplasmosis. Animal Disease Dept., University of Georgia, Tifton, Ga.

the mid-dorsal region of susceptible splenectomized calves in a cloth patch glued to the animal. All live ticks from each collection area or site were placed on one calf. Serologic and hematologic studies had indicated these test calves to be free of *A. marginale,* or other blood infections. Replete female ticks were removed from the patch as they detached and were held in separate vials in a 20 C incubator to observe their fecundity. The test calves were bled weekly for *A. marginale* examinations. Their serum was tested bi-weekly for the presence of antibodies.

Body temperatures were measured daily in calves inoculated with deer blood, from day 1 post-inoculation to termination of the trial. One 20 ml blood sample for serum extraction and one 3 ml sample collected in a vial containing the salt of ethylendiaminetetraacetic acid (EDTA) for packed cell volume (PCV), hemoglobin (Hb) determination and blood film preparation were collected from each calf. Collections were made at weekly intervals for 3 weeks, triweekly for 7 weeks and again weekly until termination of the trial 106 to 111 days post-inoculation. The susceptibility of all calves was then challenged by subcutaneous inoculation of 10 ml of blood from a known *A. marginale* carrier bovine.

Blood smears were stained by Wright's and Giesma's methods and serums were collected and handled in the same manner as reported for deer serums. All calves were 3½-5 months of age when inoculated.

RESULTS

Deer serums were tested for the presence of specific antibodies by both the CF and CT methods. Serums from two deer, one each in the Steens Mountain and Silver Lake areas, were 4+ CF positive reactors. Serum from 22 deer reacted to the CT. Four reacted at the 1+, four at 2+, five at 3+ and nine at 4+ levels. Six CT reactors were from the Steens Mountain area and eight each from the other two areas.

Examination of stained deer blood smears showed no anaplasma bodies or other blood parasites.

As shown in Table 1, amounts of blood from each deer inoculated into calves varied. In most cases, blood was inoculated subcutaneously and intravenously, with the intravenous amounts from each deer ranging from 5 ml to 15 ml. Blood from six deer was not administered intravenously because of small clots present.

No signs of systemic or local bacterial infections developed in the calves. Blood collection procedures employed were apparently adequate to prevent bacterial contamination.

Calves inoculated with deer blood were maintained in isolation during the

TABLE 1. Deer Blood Inoculated into Calves.

Calf Number	Calf age (Months)	Number deer samples inoculated	Total Volume inoculated (ml) I.V.	S.C.	Collection area
50	4	5	60	630	Steens Mountain
79	3	6	60	530	Steens Mountain
66	4	5	45	320	Ironside
46	4	5	15	310	Ironside
75	4	5	50	400	Silver Lake
76	4	5	50	400	Silver Lake

I.V. = intravenous; S.C. = subcutaneous.

trial which varied in length from 106 to 111 days. *A. marginale* was not observed in stained blood smears during this period nor did any of the calves develop signs of illness. Body temperature, PCV and Hb measurements were at no time indicative of anaplasmosis. Card tests conducted on all calf serum samples were negative. All CF tests conducted during the trial on five of the six calves were negative. Serum from calf 66 on four consecutive tests gave suspicious CF reactions (1+ and 2+). These reactions occurred on tests 12, 13, 14 and 15. The following five tests were negative.

Table 2 shows dates of challenge inoculations, dates of infection, PCV, Hb values and incubation periods. The criterion of infection utilized was the time anaplasma bodies were first observed in at least 1% of the erythrocytes. As noted in Table 2, a concomitant decrease in PCV and Hb occurred at this time. As the percentage of parasitized erythrocytes increased, PCV and Hb continued to fall. The lowest PCV was 15% and the lowest Hb 4.1g/100 ml. A substantial decrease in both PCV and Hb occurred in all calves. No deaths resulted and recovery was rapid. The incubation periods, measured from date of challenge to date of demonstrable infection, ranged between 25 and 27 days. All challenged calves developed CF and CT titers.

Table 3 indicates the species and number of live ticks collected from deer in the three areas. It also shows the number which completed engorgement when attached to the test calves. The majority of ticks placed on each test calf became

TABLE 2. Incubation Period in Calves Following Subcutaneous Inoculation of 10 ml of Blood from a Bovine **(A. marginale)** Carrier.

Calf No.	Date inoculated	PCV*	Hb. g/100 ml**	Date infection noted	PCV*	Hb. g/100 ml**	Anaplasma bodies (%)	Incubation period (days)
50	7/5	41	12.2	7/31	31	11.3	7.8	26
79	7/5	41	11.8	7/31	29	8.6	9.8	26
66	7/20	43	13.7	8/14	34	10.1	2.0	25
75	7/20	39	12.7	8/14	32	10.5	2.2	25
76	7/20	40	12.3	8/16	27	7.6	3.4	27
46	7/20	47	14.9	8/14	36	11.3	5.8	25

* Packed cell volumes determined by microhematocrit method.
** Hemoglobin values determined with Spencer Hb-meter.

TABLE 3. Live Ticks Collected from Mule Deer in Oregon (Ticks were Allowed to Refeed on Splenectomized Calves).

Location	Species	Number Female	Number Male	Number of engorged females recovered
Silver Lake	*Dermacentor albipictus*	59	6	51
Steens Mountain	*Dermacentor albipictus*	30	none	27
Ironside	*Dermacentor albipictus*	60	2	42
	Dermacentor andersoni	2	4	

active, attached, and fed to normal repletion in 7-10 days. The engorged females, after being held at 20 C, laid the usual mass of eggs which later hatched.

Test calves were observed for 90 days after the ticks fed. None developed signs of infection. Upon subsequent inoculation with blood from a known carrier of *A. marginale*, all calves were susceptible.

DISCUSSION

Two CF false positive reactions occurred among the 31 deer serum samples. Since none of the deer were infected, the CF accuracy on positive mule deer sera was not determined. The CT as conducted in this trial also proved unsatisfactory since 22 of the serum samples gave false positive reactions. Similarly, the CT and CF tests were considered unreliable when conducted on coastal blacktailed deer.[7]

The four CF suspicious recations (1+ and 2+) which occurred in calf 66 following injection of deer blood were considered nonspecific since subsequent tests were negative, parasitemia was not observed and the calf proved susceptible upon challenge.

To demonstrate that *A. marginale* would survive and remain infective for the period of time required from blood collection to inoculation, two blood samples were collected in Corvallis from a known bovine carrier. The blood samples were refrigerated in the same manner as deer blood samples collected in the field, and were shipped by bus to the most distant point from which deer blood had been shipped. The samples were re-iced in Ontario and returned to Corvallis on the next bus. Upon return, 10 ml were inoculated subcutaneously into each of two calves, numbers 46 and 66. Time between collection and inoculation was 44 hours, 8 hours longer than the longest period between collection and inoculation of deer blood. Both calves developed infection in 25 days, the shortest incubation period of any of the challenged calves.

Since all deer sampled proved to be non-infective, little information was obtained regarding the vector status of *D. albipictus*. Transmission of anaplasmosis would not be expected by these one-host ticks collected from anaplasmosis-free deer.

Only six *D. andersoni*, two females and four males, engorged when placed on a calf. Anaplasmosis did not occur in the calf subsequent to engorgement. All *D. andersoni* were collected from deer in the Ironside area. Oregon studies regarding the role of this three host tick in anaplasmosis transmission are needed since its population distribution in eastern and central Oregon coincides with the distribution of anaplasmosis.

This trial did not encompass all the anaplasmosis enzootic areas of Oregon, but the deer sampled were from enzootic areas. Those from the Ironside area were shot on a ranch where bovine losses from anaplasmosis have regularly occurred during the past several years. The method of blood collection and handling and the limited time between collection and inoculation should have precluded destruction of the infective agent had it been present. Also, the large amount of blood inoculated into calves should have been adequate to transmit the disease if one or more deer had been latent carriers. A California study demonstrated transmission from seven deer to a cow with two pooled inoculations averaging only 2.0 to 2.5 ml per donor.[2]

This trial, similar to trials conducted in Wyoming[5,9] suggests that mule deer are probably not important reservoirs of *A. marginale*. If this assumption is correct, control of anaplasmosis in parts of Oregon and in many western range states may in time become feasible. Considering the limited areas sampled in relation to the range of mule deer, however, additional studies will be necessary to definitely delineate the status of these animals in the epizootiology of anaplasmosis. Similarly, the reservoir status of antelope, bison, elk and perhaps other wild herbivora also needs clarification.

LITERATURE CITED

1. BOYNTON, W. H., W. B. HERMS, D. E. HOWELL and G. M. WOODS. 1936. Anaplasmosis transmission by three species of ticks in California. J. Amer. Vet. Med. Assoc. 88: 500-502.
2. BOYNTON, W. H. and G. M. WOODS. 1940. Anaplasmosis among deer in the natural state. Science 91: 168.
3. BRAM, R. A. and T. O. ROBY. 1970. Attempted hereditary transmission of *Anaplasma marginale* (Theiler) with *Dermacentor albipictus* (Packard). J. Parasit. 56: 620-621.
4. CHRISTENSEN, J. F., J. W. OSEBOLD, J. B. HARROLD and M. N. ROSEN. 1960. Persistence of latent *Anaplasma marginale* infection in deer. J. Amer. Vet. Med. Assoc. 136: 426-427.
5. CHRISTENSEN, J. F., J. W. OSEBOLD and J. R. DOUGLAS. 1962. Bovine anaplasmosis in the coast range area of California. J. Amer. Vet. Med. Assoc. 141: 952-957.
6. CHRISTENSEN, J. F. and D. W. McNEAL. 1967. *Anaplasma marginale* infection in deer in the Sierra Nevada foothill area of California. Amer. J. Vet. Res. 28: 599-601.
7. HOWARTH, J. A., J. O. ROBY, J. E. AMERAULT and D. W. McNEAL. 1969. Prevalence of *Anaplasma marginale* infection in California deer as measured by calf inoculation and serologic techniques. Seventy-third Ann. Proc. U.S. Animal Health Assoc.: 136-147.
8. HOWE, D. L., W. G. HEPWORTH, F. M. BLUNT and G. M. THOMAS. 1964. Anaplasmosis in big game animals: Experimental infection and evaluation of serologic tests. Amer. J. Vet. Res. 25: 1271-1276.
9. HOWE, D. L. and W. G. HEPWORTH. 1965. Anaplasmosis in big game animals: Tests on wild populations in Wyoming. Amer. J. Vet. Res. 26: 1114-1120.
10. KUTTLER, K. L., R. M. ROBINSON and T. E. FRANKLIN. 1968. Serological response to *Anaplasma marginale* infection in splenectomized deer *(Odocoileus virginianus)* as measured by the complement-fixation and capillary-tube agglutination tests. Proc. 5th Nat. Anaplasmosis Conf. (U.S.A.): 82-88.
11. OSEBOLD, J. W., J. F. CHRISTENSEN, W. M. LONGHURST and M. N. ROSEN. 1959. Latent *Anaplasma marginale* infection in wild deer demonstrated by calf inoculation. Cornell Vet. 49: 97-115.
12. OSEBOLD, J. W., J. R. DOUGLAS and J. F. CHRISTENSEN. 1962. Transmission of anaplasmosis to cattle by ticks obtained from deer. Amer. J. Vet. Res. 23: 21-23.

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